DETAILED ACTION

Priority

Acknowledgment is made of applicants' claim for priority of U.S. provisional application 60/510231, filed October 9, 2003. A claim for priority cannot be based on said application, since the United States application does not provide support for the present broad claim 1, for example.

Status of Claims

Calims 1-31 are pending in the application.

Claims 11-31 withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claims.

Claims 1-10 are under examination.

Withdrawn Objection/rejection

In view of the amendments to the abstract and specification, the objection to the specification is withdrawn. Also, the rejection under 35 USC 112, 2nd paragraph-in part has been overcome.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 112

Claims 1-10, as amended, are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention for reasons of record as reiterated below.

The specification fails to provide an adequate written description of the claimed method for which the components use in the method has no identifying or characterizing features. The claim method encompasses a huge number of components for which no corresponding written description is provided in the disclosure. The specification describes a single species of a reporter protein, Split-Trp. The disclosure does not indicate whether the experimental conditions or other parameters employed for the species could be extrapolated or applicable for the huge scope of the genus components in the method. At the time of applicants' invention, the art has recognized that in generating new split-protein sensors it is critical to identify suitable fragments that can reconstitute a native-like and active

protein. The success of such selection of the fragment sites can depend on numerous factors such as that the two fragments have to efficiently fold into quasi-native but only in the presence of specific peptides and the fragments are not readily degraded in vivo.

A "written description of an invention involving a chemical **genus**, like a description of a chemical **species**, requires a precise definition, such as by structure, formula [or] chemical name of the claimed subject matter sufficient to distinguish it from other materials". University of California v. Eli Lilly and Co. 43 USPQ 2d 1398, 1405(1997), quoting Fiers V. Revel, 25 USPQ 2d 1601m 16106 (Fed. Cir. 1993).

Applicant, at the time of filing, is deemed to have not invented species sufficient to constitute the genus by virtue of having disclosed a single species when … the evidence indicates ordinary artisans could not predict the operability in the invention of any species other than the one disclosed. In re Curtis, 354 F.3d 1347, 1358, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004).

One may not preempt an unduly large field by the expedient of making broad prophetic statements in the specification and claim unless the accuracy of such statements is sufficiently

supported by well-established chemical principles or by sufficient number of examples

[This rejection can be obviated by reciting in the independent claim the split-protein sensor of claim 10.]

Response to Arguments

Applicant's arguments filed 10/21/09 have been fully considered but they are not persuasive.

Applicants acknowledge that it is true that the identification of new split-protein sensors is difficult since selection of suitable fragmentation sites depends on various factors and that pre-selected fragmentation sites might not work as expected. But argue that this is the problem that the claimed invention overcomes. The invention provides a system where a large library of randomly created fragmentation sizes in a reporter protein is generated and where this library is then subsequently screened for fragment pairs that can reconstitute a native-like and active protein in vivo. The invention therefore provides an efficient method to identify such fragments, without having to first identify possible suitable fragments through sequence or conformational analysis. Thus, the skilled artisan now for the first time may well predict the operability of the invention for any protein, as required by Fed. Cir. in In re

Curtis, 354 F.3d 1347, 1358, 69 USPQ2d 1274, 1282 (Fed. Cir. 2004).

In reply, a single disclosure of a protein-protein interaction would hardly be considered a predictable operability for any kind of protein. Protein-protein interaction is highly unpredictable, especially when method is alleged to be novel. Applicants have not proffered any evidence, except for the above statements, that other species encompassed by the huge genus claim would operate based on the single described species.

Applicants assert that the generation of libraries with random sequences is well known in the art. An exemplary method to generate such a library is given in the specification in paragraph [0024]. This exemplary method uses standard DNA manipulation techniques known in the art, such as PCR amplification, PCR overlap extension and molecular cloning techniques. These techniques are all very well established and optimization of the protocols is routine work for a person skilled in the art. A person skilled in the art is also able to create such a library for a large number of other genes than Trp, since the standard DNA manipulation techniques cited above are well established in the art.

In reply, generation of a library with random sequences is well known in the art but only for a given or known protein structure. However, for an unnamed or unstructured protein, it is not apparent as to how a skilled artisan would proceed to create a library, lacking any direction, description and guidance in the instant specification. There is no description in the specification as to the number of residue(s) randomized, the length of the random sequences(s) and/or the location of the sequence in the protein that can be randomized. This is made more compelling in view of the assertion that the method is novel.

Claim Rejections - 35 USC § 112, 2nd paragraph

Claims 1-10, as amended, are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for reasons of record as reiterated below only for the MAINTAINED REJECTIONS.

5. The term "close" in claim 1 is a relative term which renders the claim indefinite. The term is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in

the art would not be reasonably apprised of the scope of the invention. It is not clear as to the measure of such distance such that it is considered “close” to each other.

Response to Arguments

Applicants argue that the term "close proximity" has been altered to "close spatial proximity so as to allow reconstitution of both first and second subdomains into an active protein" so as to more clearly specify the measure of the required distance.

In response, even with the addition of the term “spatial”, the claim “close spatial proximity” is still vague and indefinite. What is the spatial arrangement or proximity of the subdomains to be considered “close”? Is the spatial proximity relative to one another in reference to its tertiary structure? Given no name or structure for any of the components, the "close spatial proximity” is indefinite.

5. Non-sequitur for “the transcription", claim 8: "the wild-type length", "the original N-and C-termini”, “the vector”, “the plasmid at the 3’ and the 5’ends and “the zippers” all in claim 10.

Response to Arguments

Applicants assert that in claim 8, "...the transcription..." has been clarified by reciting "...the transcription...into a polypeptide". Transcription of a gene into a polypeptide is well known and therefore this new formulation should be unambiguous. In claim 10, "the wild type length" has been changed to "the wild type length of TRPI"; "the original N- and C-termini" has been clarified by reciting "the original N- and C-termini of TRP 1"; "the vector" has been replaced by the "the expression vector" which is defined in the third step of the method of claim 10; and "the zippers" have been replaced by "the leucine zippers" which are defined in the same step of claim 10.

In reply, the amendments to e.g., claim 8 has not overcome the rejection since "**the** transcription" is not recited in the series of dependent claims, 7 and 4 which all ultimately depend on independent claim 1. The (which implicitly refers to -said-) is not recited in the independent claim 1. Transcription might be known in the art however, the base claim does not recite said

transcription. [It is suggest that applicants change "the" to e.g., ---a---.]

Claim 4 is rejected under 35 U.S.C. 112, 2nd paragraph as follows:

Claim 4 does not further limit claim 1 since this limitation has been added to the currently amended claim 1. It is suggested that applicants delete said claim 4.

Claim Rejections - 35 USC § 102

Claims 1-9, as amended, are rejected under 35 U.S.C. 102(e) as being anticipated by Balint, Robert F., et al (20040038317).

For claim 1, Balint discloses throughout the patent at e.g., the abstract fragment pairs of a Class A β-lactamase (TEM-1 of E. coli) that depend for their functional reassembly into the parent protein on the interaction of heterologous polypeptides or other molecules which have been genetically or chemically conjugated to the break-point termini of the fragment pairs. In addition, methods are provided for identifying fragment pairs that will optimally reassemble into a functional parent protein. Balint discloses at e.g., paragraph [0024] FIG. 9, vectors and protocol for construction of a multiplex protein-

protein interaction library using interaction-dependent β-lactamase fragment complementation systems. Expressed sequence (ES), i.e., random-primed cDNA libraries, is subcloned into phagemid vectors for expression as fusions to the β-lactamase .alpha. and .omega. fragments, via the flexible linker (Gly.sub.4Ser)3. Balint discloses at paragraph [0029] that the first interactor domain is a known or unknown protein or protein fragment that binds directly or indirectly to a second target interactor domain that is an unknown protein or protein fragment and either or both the first and second interactor domain can be a member of a library. When combining the first and second oligopeptide sequences, the reconstitution of the N-terminal and C-terminal fragments into the marker protein requires the prior interaction of the first and second interactor domains.

For claim 2, Balint discloses at e.g., paragraph [0047] enzymes which could be activated to hydrolyze chromogenic substrates only upon binding to target analytes could form the basis of assays for those analytes of unparalleled sensitivity and convenience. Such assays would be homogeneous, requiring no manipulations other than the mixing of two components namely than enzyme and substrate, with a biological specimen, in which

the presence of the analyte is then quantitatively indicated by the rapid development of color.

For claims 3 and 6, Balint discloses at paragraph [0037] methods of identifying functional fragment pairs of a marker protein of interest that involves preparing a multiplicity of fragment pair members with break-point termini within a solvent exposed loop or a flexible loop defined by tertiary or secondary structure analysis to obtain a fragment pair library. The fragment pair members are expressed in a multiplicity of host cells, and the host cells exhibiting the directly detectable signal associated with the marker protein of interest is isolated as indicative of containing fragment pair members that functionally reconstitute the marker protein. Plasmids containing expression cassettes coding for the fragment pair members are then sequenced to identify functional fragment pairs. To aid in the identification of functional fragment pair members of a marker protein of interest, the fragment pair members can be expressed as fusion proteins with interactor domains known to bind to each other, such as the fos and jun transcription factors that associate through a leucine zipper interaction. The sequences encoding the hetero-dimerizing

helices of the fos and jun transcription factors are sufficient to use as effective interactor domain.

For claim 5, Balint discloses at paragraph [0041] the Class A β-lactamases are comprised of two domains. One domain, alpha, omega is made up of N-terminal and C-terminal sequences, which form an anti-parallel two-helix bundle packed against a flat 5-stranded β-sheet.

For claims 3, 4, 8, 9, Balint discloses at paragraph [0034] appropriate host cells include both eukaryotic cells, such as mammalian, yeast and plant cells, and prokaryotic cells, such as bacterial cells. Expression vectors can be constructed which contain a promoter to direct transcription, a ribosome binding site, and a transcriptional terminator. Examples of regulatory regions suitable for this purpose in E. coli are the promoter and operator region of the E. coli tryptophan biosynthetic pathway as described by Yanofsky (1984) J. Bacteriol., 158:1018-1024 and the leftward promoter of phage lambda (P.lambda.) as described by Herskowitz and Hagen, (1980) Ann. Rev. Genet., 14:399-445. See Sambrook, et al (In Molecular Cloning: A Laboratory Manual, 2.sup.nd Ed., 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor) for a description of other prokaryotic expression systems.

Response to Arguments

Applicants assert that the sequence of a known reporter protein is fragmented into two subsequences of random lengths and each subsequence is then coupled to a nucleotide sequence coding for proteins or peptides which are known to interact with each other resulting in sequences coding for two fusion proteins. The method as described in Balint relies on subdomains of a reporter protein with a defined length and which are known to be able to reconstitute an active-like protein. These fragments are coupled to two proteins or peptides to find out whether these interact with each other. Balint proposes at [0037] a method to identify functional fragment pairs of a reporter protein. The major difference to the method of the present invention is that in Balint suitable break points within solvent exposed loops have to be selected beforehand following a tertiary or secondary structure analysis. The method of the present invention does not necessitate any analysis of protein structure but enables the generation of a library with random fragment pairs of a reporter protein. This library may then be screened for fragment pairs which reconstitute the function of the reporter protein in vivo. The present method considerably speeds up the procedure to find suitable fragment pairs and does

also enable the generation of split-protein reporter systems from proteins where the structure has not yet been analyzed.

In reply, it is not seen how a library can be created from an unnamed or unstructured or unknown (i.e., "not yet analyzed" as stated above) reporter protein. Furthermore, the preamble also recites a suitable fragmentation site even for an unnamed or structurally undefined reporter protein. There is nothing in the claims to preclude analysis of the secondary or tertiary structure of the reporter protein such that the precise fragmentation sites are made. Accordingly, the specific process steps of Balint with a known reporter protein sequence fully meet the broad claim process steps. The method of reconstituting fragments of a parent (original) protein to produce again the active reporter protein from which the fragments are obtained is well-practiced in the art at the time of the invention. There is nothing new and novel about the broad claim process steps.

Claims 1-9, as amended, are rejected under 35 U.S.C. 102(b) as being anticipated by Johnsson et al (PNAS) for reasons of record as set forth below.

Johnsson et al discloses e.g., at page 10340, the abstract an assay method for in vivo protein interactions. Protein fusions containing ubiquitin are rapidly cleaved in vivo by ubiquitin-specific proteases, which recognize the folded conformation of ubiquitin. When a C-terminal fragment of ubiquitin (Cub) is expressed as a fusion to a reporter protein, the fusion is cleaved only if an N terminal fragment of ubiquitin (Nub) is also expressed in the same cell. This reconstitution of native ubiquitin from its fragments, detectable by the in vivo cleavage assay, is not observed with a mutationally altered Nub. However, if Cub and the altered Nub are each linked to polypeptides that interact in vivo, the cleavage of the fusion containing Cub is restored, yielding a generally applicable assay for kinetic and equilibrium aspects of in vivo protein interactions. This method, termed USPS (ubiquitin-based split-protein sensor), makes it possible to monitor a protein-protein interaction as a function of time, at the natural sites of this interaction in a living cell.

Johnsson discloses at e.g., page 10342, Fig. 2, a fusion construct containing some of the following elements: (i) a Ub moiety, either wild-type (construct I) or bearing single-residue replacements at position 13 (constructs II-IV). (ii) A Ub moiety containing the 68-residue insertion (denoted as Ste6) derived

from the cytosolic region of S. cerevisiae Ste6 between its transmembrane segments 4 and 5). The insertion was positioned after residue 36 of Ub (construct V). (iii) A Ub moiety bearing both the above insertion and a single-residue replacement at position 13 (constructs VI-VIII). (iv) A C-terminal fragment of Ub (Cub, residues 35-76) bearing the 32-residue, Ste6- derived sequence at its N terminus (construct IX). (v) The same fusion whose N terminus was extended, via the linker sequence Gly-Glu-lle-Ser-Thr, with the 47-residue homodimerization motif ("leucine zipper") of S. cerevisiae Gcn4 (18-21) (residues 235-281 of Gcn4) (construct XIV). (vi) An N-terminal fragment of Ub (Nub, residues 1-37) bearing the wild-type Ub sequence or a single-residue replacement at position 13 and a C-terminal extension containing the linker sequence Gly-Gly-Ser-Thr-Met followed by the leucine zipper of Gcn4 (constructs X-XIII). (vii) Mouse DHFR bearing a C-terminal ha epitope (14) (denoted as DHFR in this diagram and as dha in the text and in Figs. 3 and 4). Johannson further discloses at e.g., page 10341, col. 1 the mutations of UB at position 13 (Ile) being replaced by Val, Ala or Gly (i.e., a library of residues). See also the Materials and methods section at page 10340, col. 1 up to the Results section, page 10343 which describes the specifics of the method.

Accordingly, the method of Johnsson which describes the method for a species, ubiquitin-split sensor, fully meets the broad claim method wherein none of the compounds in the method is defined or with features distinguished from that of the prior art.

Response to Arguments

Applicants argue that Johnson et al (PNAS) similarly describes a method to determine protein-protein interactions by the way of restoration of ubiquitin mediated degradation. Ubiquitin is split in two fragments, which will reconstitute a native-like protein structure only when fused to two proteins or peptides interacting with each other. Johnson does not describe any method which is suitable to identify suitable reporter protein fragments. On the contrary, the method described relies on reporter protein fragments of a known length which are fused with proteins or peptides to be screened for interaction. The method of the claimed invention however relies on protein or peptides which are known to interact to screen a library with fragments of a reporter protein having random lengths to identify fragment pairs being able to reconstitute a native-like protein.

In response, applicants' arguments are not commensurate in scope with the claims, which do not recite for any length for the reporter protein. Be that as it may, the known length of Johnson therefore fully meets the broad claim having an unknown (random) length i.e., the known length falling within the scope of the broad random length fragments.

Claims 1-4 and 7-9, as amended, are rejected under 35 U.S.C. 102(b) as being anticipated by Luger et al (Science) or Eder et al (Biochemistry) for reasons of record as repeated below.

Luger et al discloses throughout the article at e.g., page 207, Figs. 1, 2 and col. 2, a method of fragmenting phosphoribosylanthranilate isomerase (TrpI) inserting amino acid residues that recombine the fragments.

Eder et al basically discloses, throughout the article at e.g., page 3618, the same method as Luger above. See the specifics of the method at Experimental Procedures at page 3618, col.2 up to page 3620 including the RESULTS section, up to e.g., page 3622.

Response to Arguments

Applicants assert that Luger et al (Science) and Eder et al (Biochemistry) also rely on specific selection of fragmentation sites. Therefore, in contrast to the claimed invention, both Luger and Eder do not disclose the use of randomly created subsequences of the DNA encoding for subdomains of the protein. Therefore, claim 1 is novel over these references. Neither of these publications proposes a system to generate random length fragments of a protein or the generation of a library.

In reply, the preamble of claim 1 recites for selection of a suitable fragmentation sites i.e., specific selection of fragmentation sites as that of Luger or Eder.

Accordingly, the specific process steps of Luger or Eder that employs specific components for the process trp1 fully meets the broad process steps of an unnamed reporter protein of e.g., claim 1.

Claims 1-10, as amended, are rejected under 35 U.S.C. 102 (a) as being anticipated by Tafelmeyer et al (Chemistry and Biology, 5/2004) for reasons set forth in the last Office action as repeated below.

Tafelmeyer discloses at e.g., page 681, Summary section a combinatorial method for generating a split-protein sensor which allows for the detection of protein-protein interactions in vivo. The method is applied toward Trp1p split protein.

Claim 2 growth assay is disclosed at page 681, col. 2, Results and Discussion.

Therefore, the specific process steps of Tafelmeyer fully meets the broad claimed method without any distinguishing features for the components e.g., reporter protein as claim in the method. [Please note that the priority document with a filing date of 10/9/03 does not provide support for the broad claim method of claims 1-9. Therefore, applicants are accorded the priority date as of the PCT application with a filing date of 10/8/04.]

Response to Arguments

Applicants argue that Tafelmayer et al was published in 2004 (see first page of the document) by the inventors less than a year before the filing date of the international application (October 8, 2004). Therefore, that document is not prior art

under 35 U.S.C. 102(b), even if the prior U.S. provisional application's date is disregarded.

In reply, the rejection is based on 102 (a) not 35 USC 102 (b) as set forth above.

(Please note that the instant inventors are different from the authors of the published article.)

No claim is allowed.

Allowable Subject Matter

Claim 1 would be allowable if rewritten or amended to include claim 10 and overcome the rejection(s) under 35 U.S.C. 112, 2nd paragraph, set forth in this Office action and submission of an In re Katz declaration to overcome the 35 USC 102(a) rejection over Tafelmayer.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS

of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

This application contains claims 11-31 drawn to non-elected inventions. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to TERESA WESSENDORF whose telephone number is (571)272-0812. The examiner can normally be reached on flexitime.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/TERESA WESSENDORF/
Primary Examiner, Art Unit 1639